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Bioassay of Acephate-Treated Foliage on Three Instars of the Douglas-fir Tussock Moth

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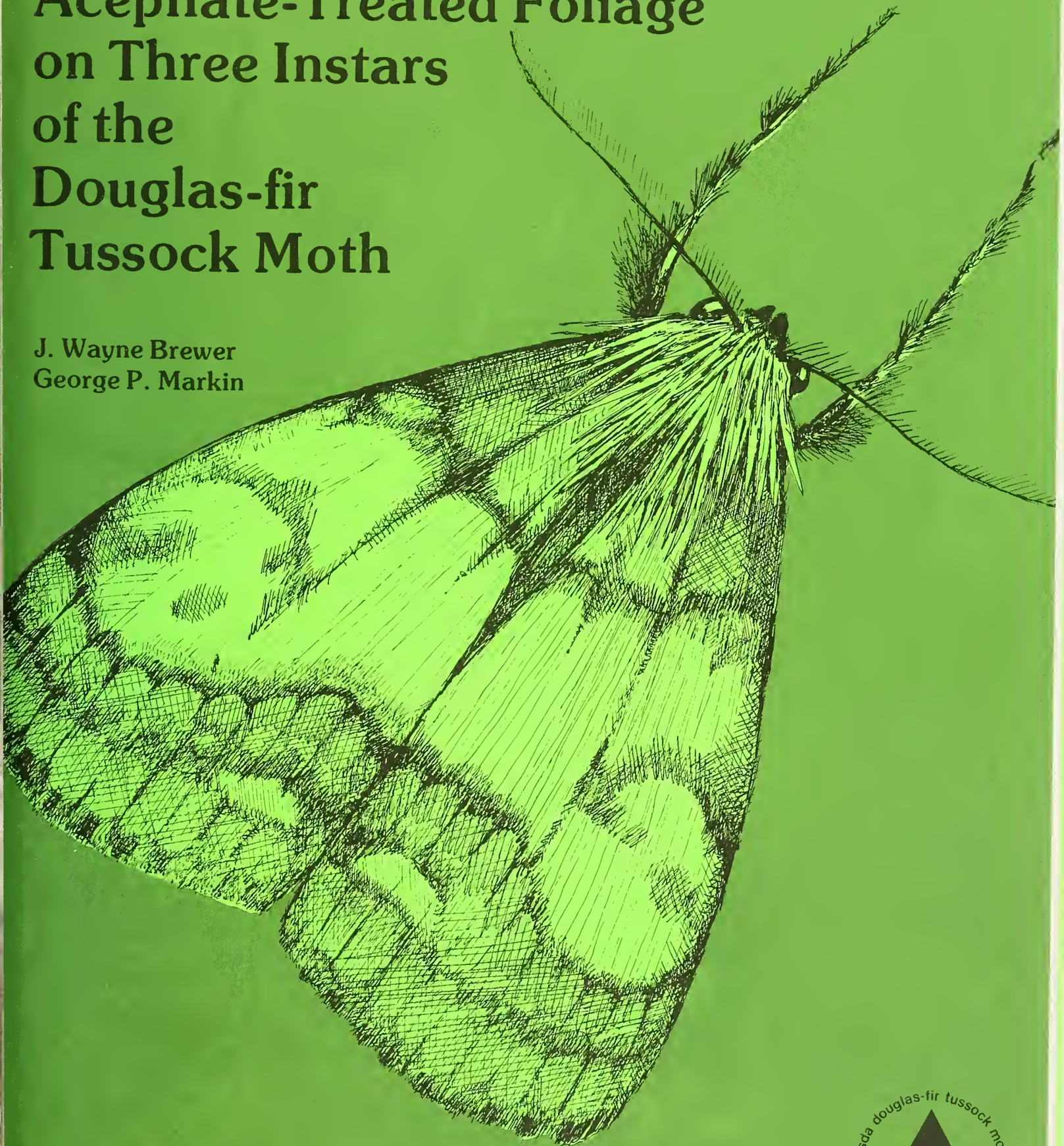

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BIOASSAY OF ACEPHATE-TREATED FOLIAGE ON THREE INSTARS OF THE DOUGLAS-FIR TUSSOCK MOTH

Reference Abstract

Brewer, J. Wayne and George P. Markin.
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KEYWORDS: Bioassay, acephate, insect damage control (forest), Douglas-fir tussock moth, *Orgyia pseudotsugata*.

RESEARCH SUMMARY

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Field application of acephate was made on a series of 16-acre (6.5 ha) plots of Douglas-fir *Pseudotsuga menziesii* var. *glauca* (Beissn.) Franco, and grand fir, *Abies grandis* (Dougl.) Lindl., at the rate of 1 lb AI/acre (1.12 kg/ha) in central Washington. Bioassays of first, second, and third instars of *Orgyia pseudotsugata* (McDunnough) on foliage from treated and check plots indicated that acephate caused a significant increase in larval mortality. The highest mortality, 88 percent, occurred in first instars tested 1 day after treatment. Mortality decreased with older instars and with time after treatment. Mortality trends were similar for the three instars over the 21-day post-treatment sampling period.

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Introduction

Acephate is an organophosphate insecticide with systemic activity (Werner 1974) and low mammalian toxicity. It appears to be effective against a variety of forest pests and yet seems more compatible with environmental concerns than many other chemical insecticides (Brewer and O'Neal 1977). Acephate has been field tested against gypsy moth, *Lymantria dispar* (L.), elm spanworm, *Ennomos subsignarius* (Hubner) Doane and Dunbar (1973), elm leaf beetle, *Pyrrhalta luteola* (Muller) Brewer (1973), eastern spruce budworm, *Choristoneura fumiferana* (Clemens) Hopewell and Nigam (1974) and a looper *Lambdina athasaria athasaria* (Walker) Cameron and Mastro (1975). Neisess et al. (1976) demonstrated that acephate gave good control of second, and third instars of the Douglas-fir tussock moth, *Orgyia pseudotsugata* (McDunnough)[1] in small plots. We wanted to determine if field application of acephate would cause different levels of mortality of the first three instars of the Douglas-fir tussock moth. We also wanted to study the residual effect of acephate on these instars. We used laboratory bioassays of field-treated foliage in the test because we could better control developmental stage of test larvae than under field conditions. Also, this approach allowed us to determine the residual activity on larvae introduced at specified time intervals after treatment but still incorporated the normal effects of chemical weathering that occur in the field.

Materials and Methods

The study was conducted within the framework of a large aerial application test of various types of insecticides located ca. 25 miles (40 km) northeast of Ellensburg, (Kititas Co.) Wash., in the Swauk Creek Drainage of the Wenatchee National Forest. The treatments in this study were acephate 75 percent SP and an unsprayed check. The dosage was 1 lb AI (1.12 kg) in 1 gal (3.8 liter) H_2O with 3.785 g of fluorescent dye (Rhodamine B Extra S (GAF))[2] added for deposit assessment. The material was applied at the rate of 1 gal/acre (3.8 liter/ha).

EXPERIMENTAL DESIGN

Six plots, 16 acres (6.5 ha) each, were established with each plot containing a 15-tree cluster designated for sampling and spray deposit analysis. The plots were separated by a minimum of 1 kilometer. Sample trees were either open-grown Douglas-fir, *Pseudotsuga menziesii* var. *glauca* (Beissn.) Franco, or grand fir, *Abies grandis* (Dougl.) Lindl., 30 to 50 ft (9 to 15 m) in height. Plot corners and boundaries were marked with brilliantly colored plastic markers 18 X 60 in (45.7 X 152.4 cm) pulled into treetops with line shot over the trees with a crossbow or a line-throwing gun (Maksymiuk 1975). In some cases, plot corners were designated by markers dropped from a helicopter.

Three replicates of each of two treatments were used, with plots assigned at random. Check plots were not sprayed nor were corners marked, but sample trees were designated.

APPLICATION

The acephate formulation was applied to the three treated plots by a Hiller UH-12E helicopter equipped with eight spinning-disk atomizers mounted on an 18-ft (5.5-m) support bracket. The atomizers were a modified version of the Balls Turbear spinning nozzle (Boving et al. 1971) consisting of 12 plates, 4 inches in diameter (10 cm), driven by an

[1] Lepidoptera: Lymantriidae.

[2] General Aniline & Film Corp.

electric motor at 9000 rpm. The atomizers were fed from two interconnected 10-gal (37.8-liter) stainless steel tanks pressurized at 40 lb/in^2 (2.8 kg/cm^2) by compressed nitrogen. The aircraft treated one 16-acre (6.47-ha) plot/flight with a reserve of 4 gal (15.1 liter) of material remaining in the system.

Applications were made during the early morning of June 15. During applications, the temperature ranged from 44° to 52°F (6.6° to 11°C) and the RH from 85 to 100 percent. There was no measurable wind at plot one and less than 0.5 mph (0.8 km/h) at the other two sprayed plots. There was no measurable precipitation on the plots until a brief rain shower occurred on June 18 and again on June 19. There was no further precipitation during the sampling period.

During the operation, the pilot oriented himself over the plot using the four treetop corner markers and directions from a radio-equipped ground observer. Spraying started on the downwind side of the plot and progressed upwind with swaths spaced 50 ft (15 m) apart at 35-50 ft (11-15 m) above the treetops.

EVALUATION

Spray Deposit Assessment.--Spray deposit was sampled at the forest floor of sprayed plots with 6 in square (15.2-cm) aluminum plates and 4 X 5 in (10.2 X 12.7 cm) white Kromekote cards according to the procedure of Maksymiuk and Orchard (1975). Two plates and one card were placed in small openings adjacent to each sample tree. Foliage samples for deposit analysis were collected from sample trees within 3 hours of spraying. One 15-in (38.1-cm) branch sample was cut from each of four cardinal directions from midcrown. Plates, cards, and foliage were transported to the laboratory for analysis.

Amounts of spray deposit on the foliage and aluminum plates were determined by a modification of the fluorometric analysis reported by Yates and Akesson (1963), using a Turner Model #430 Spectrofluorometer. For foliage samples, 5 g subsamples of air-dried foliage were taken at random from each branch sample. Spray deposit was eluted from needles by washing them 20 min in 50 ml of H_2O with the addition of 3 drops of Laboratory Aerosol (10 percent AI dioctyl solium sulfosucciinate). Deposit was eluted from aluminum plates with another 10 ml of the aforementioned solution. Deposit from the plates was converted to gal per acre and from foliage to ml of formulation per g of air-dried foliage.

Atomization and drop densities of the spray deposit on the Kromekote cards were determined with a Quantimet 720 Image Analyzer.

BIOASSAY SAMPLES

A large foliage sample ca. 36 in (90 cm) in length was removed from each of four sides of each of the 15 designated sample trees for all three replications of the treatments 1 day prior to insecticide application and at 1, 7, 14, and 21 days post-treatment. Each of the three composite subsamples for use in the larval bioassay was made by taking one 12-, to 15-in (30-, to 40-cm) branchlet from each of the four large foliage samples. Each composite subsample was placed in an 18 X 6 in (45 X 15 cm) perforated plastic bag. Foliage was stripped from the basal 4 to 6 in (10 to 15 cm) of the branches and the bag sealed at the base of the foliage so that the stripped branches protruded. The branch ends were placed in water-filled vials to reduce desiccation.

BIOASSAY

At the laboratory, 20 and later 10 Douglas-fir tussock moth larvae of first, second or third instar, as appropriate, were introduced into the plastic bag containing the foliage samples. At the end of 7 days, mortality was determined for that particular sample.

Larvae were introduced into the sample bags as soon as possible, usually on the day following collection. Foliage samples were stored temporarily in a cold room at 40°F (4.5°C) prior to introduction of the larvae. Because of lack of suitable larvae, it was frequently necessary to store foliage in the cold room for up to 23 days prior to introduction of the larvae (table 1), which undoubtedly altered insecticide potency and larval survival rates.

STATISTICAL ANALYSIS

Mortality data were subjected to analyses of variance, covariance, and regression. Adjustment of the mortality data from the various treatments was performed using an analysis of covariance with the pretreatment counts as the covariant which allowed the results to be compared directly. Thus data on pretreatment mortality are not presented. Differences were considered significant at the 0.05 probability level.

Results and Discussion

DEPOSIT ASSESSMENT

Spray coverage varied considerably among the three treated plots (table 2). Mean deposits ranged from 0.130 to 0.599 gal/acre. Substantial variation also occurred in spray droplet numbers with a range of 16.2 to 39.4 drops/cm^2. Deposit trends from foliage samples were similar to those from aluminum plates at ground level. In general, droplet size (VMD) varied inversely with ml per g of spray from foliage and gal/acre as determined from aluminum plates.

EFFICACY - DEPOSIT

Mean larval mortality (unadjusted) shows that mortality was highest in plots that received the highest deposits as measured in gal per acre and drops per cm^2, and lower where less material was deposited (table 1). Thus, it appears

Table 1--*Mean percent larval mortality by plot with gal per acre of acephate at ground level as determined from aluminum plate samples.*[1] *Kititas County, Washington, 1975*

Plot	Time after treatment (days)[2]					
	gal/ acre	-1[3]	1	7	14	21
Foliage storage[2] time (days)		0	7	23	21	21
Treated						
Plot I Liberty Rd. 2	0.218	6.6	70.3	30.6	20.6	18.0
Plot II Mill Creek 2	.130	3.7	69.0	21.0	11.0	21.3
Plot III Iron Creek 3	.599	10.0	95.3	27.3	23.0	24.0
Mean	.316	6.8	78.2	26.3	18.2	21.1
Check						
Plot I Iron Creek 2	--	8.3	9.7	8.7	13.3	14.7
Plot II Mill Creek	--	6.3	4.7	9.0	13.3	13.7
Plot III Blue Creek	--	7.3	9.0	6.0	7.7	10.0
Mean	--	7.3	7.8	7.9	11.4	12.8

[1]Data from 3 larval instars are combined.

[2]Lack of suitable larvae made it necessary to store foliage at 40°F prior to larval introduction.

[3]Day -1 counts were made 1 day prior to insecticide application.

Table 2--*Acephate spray deposit recovered from sample tree foliage and from ground level plots. Kititas County, Washington, 1975*

Treatment (plot)	Spray deposit			
	ml/g foliage	Ground level		
		Grams/acre	Drops/cm^2	VMD[1] (μm)
Acephate				
I Liberty Rd. 2	0.210	0.218	16.2	205
II Mill Creek	.125	.130	17.8	222
III Iron Creek 3	.285	.599	39.4	172
Average	.207	.316	24.5	200

[1]VMD = Volume mean diameter, the drop diameter that satisfies the condition that half the spray volume is of drops larger and half is of drops smaller than the VMD.

likely that some of the variation in larval mortality was a result of variation in the amount of material reaching the plots. Mortality from two plots (Liberty Rd. 2 and Mill Creek 2) was not as high as levels reported previously by Neisess et al. (1976), for acephate on Douglas-fir tussock moth larvae. That test was not directly comparable to this one, however, because Neisess et al. (1976), applied the material directly to field populations of the tussock moth, providing contact insecticidal action on the larvae, which was not the case in our present study. Also, in our bioassay method, larvae were not introduced until 24+ h after application; so some insecticidal activity was presumably lost prior to the first larval introduction. These test differences, in addition to the low insecticide deposit levels from those two plots, probably account for the low mortality compared with that reported by Neisess et al. (1976).

EFFICACY - TIME

Adjusted mean mortality data combined for all 3 instars throughout the sampling period were 37.5 percent in bioassays of foliage from treated plots compared with 8.8 percent for untreated checks (table 3). The highest level of mortality occurred on foliage samples collected at day 1 after insecticide application when 77.2 percent of all larvae in the bioassay, regardless of instar, were killed, compared with 7.6 percent for larvae on untreated foliage. Thereafter, mortality decreased rapidly. At 7 days post-treatment, mean larval mortality from treated foliage was 28.4 percent compared with 8.1 percent for untreated material. Differences in larval mortality between treated and untreated foliage decreased further at the 14, and 21 day post-treatment sampling periods. These data indicate that, under our study conditions, larval mortality beyond 7 days after treatment was not significantly greater than would be expected under natural conditions. Rain showers that occurred on days 3 and 4 after treatment probably washed some insecticidal deposit from the foliage. Storage of foliage in the cold room (table 1) prior to larval introduction undoubtedly was a factor in the loss of insecticidal activity. The considerable residual activity, in spite of storage, suggests that effects might have lasted longer if foliage had not been stored.

It appears from the field and laboratory conditions in our study, therefore, that the residual activity of acephate is less than 14 days against the three instars of the Douglas-fir tussock moth we tested. Cameron and Mastro (1975) reported little activity of acephate on a hemlock looper in Pennsylvania after

Table 3--*Adjusted percentage mortality means*[1] *of acephate treatment vs. check for 3 instars of Douglas-fir tussock moth. Kititas County, Washington, 1975*

Treatment	Time after application (days)				
	1	7	14	21	Mean
Acephate					
1st instar	87.8	40.1	29.2	35.6	[2]48.2
2d instar	77.0	29.0	17.7	19.5	35.8
3d instar	68.1	17.4	7.4	6.7	24.9
Means[1]	77.2	28.4	17.7	20.7	37.5
Check					
1st instar	16.9	5.7	19.4	24.3	16.6
2d instar	2.3	13.6	11.5	12.6	10.0
3d instar	2.1	4.2	2.2	3.6	3.0
Means[1]	7.6	8.1	11.8	13.6	8.8

[1]Means are adjusted to account for differing mortality on pretreatment foliage samples from the various plots using this mortality as a covariant. Since various means are adjusted according to different regression lines, arithmetic means of data presented are not necessarily equal to adjusted means.

[2]Mean instar mortality without regard to time after treatment.

1 week. Nigam (1975), using a laboratory bioassay technique for acephate, reported that mortality of spruce budworm larvae dropped from 95 percent immediately after treatment to 15 percent 5 days later. Thus it would seem that application of acephate must be closely timed to coincide with insect activity because of the short residual action of the material.

EFFICACY - INSTARS

Adjusted mean larval mortality data for each of the first, second and third instars, combined for all sampling periods, were significantly different in bioassays of treated than untreated foliage (table 3). Thus, treatment with acephate against any of the three instars tested could be expected to produce significant mortality compared with check plots. The highest mean mortality level (48.2 percent) for all four sampling intervals occurred when first instars were placed on treated foliage. Thereafter, mean mortality declined to 35.8 percent for second, and 24.9 percent for third instars, compared with 16.6, 10.0, and 3.0 percent mortality for first, second, and third instars, respectively, on check foliage. The significant interaction between treatment and instar indicates that expected mortality resulting from acephate treatment decreases as the insect goes from first to second to third instar (table 3). Thus, application of acephate against first instars should produce the greatest mortality regardless of time of sampling, at least up to 21 days post-treatment.

TIME-INSTAR INTERACTION

Table 3 also presents adjusted mortality means from treated plots and untreated controls for the three instars at four time periods after treatment. The instar X time interaction was not significant indicating that the general mortality trends for the three instars were similar over the 21-day postspray sampling period.

In the acephate treated plots, the greatest change in mortality over successive time periods occurred between days 1 and 7 after treatment. In the check plots, mortality varied somewhat but was generally similar throughout the sampling period.

GENERAL COMMENTS

The results of this study demonstrate that acephate gave control of all three instars of the Douglas-fir tussock moth tested. The fact that the level of control was not as high as reported by some previous investigators may be a result of some of the limitations of using laboratory bioassays on field-treated foliage and the special problems we encountered in this particular test. However, this method does offer a valuable approach to insecticide evaluation. Most important, the investigators are not dependent on field populations of the pest insect. Residual activity of the insecticide also is easily measured, since new insect groups may be introduced at any selected time interval after treatment. In addition, counts may be made more easily, and perhaps more accurately, under laboratory conditions than in the field.

In laboratory bioassays of field-treated foliage, pretreatment tests are of considerable value. These bioassays may be used as an indicator of foliage quality, which may differ among plots, perhaps as a result of earlier or unknown insecticide applications. In addition, pretreatment bioassays may provide a baseline for determination of changes in control plots during the test, perhaps as a result of drift from treated plots. Some of the limitations of laboratory bioassays for evaluation of field applied insecticides have been previously noted, i.e., the lack of contact action on the target insect, and the loss of the initial, and possible high, mortality at the time of application. Other limitations are that the pest insect must be amenable to laboratory culture and the cost is considerable for facilities and personnel for the insect-rearing process.

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